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Hybridization Probes for Conventional DNA Fingerprinting Used as Single Primers in the Polymerase Chain Reaction To Distinguish Strains of *Cryptococcus neoformans*

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In conventional DNA fingerprinting, hypervariable and repetitive sequences (minisatellite or microsatellite DNA) are detected with hybridization probes. As demonstrated here, these probes can be used as single primers in the polymerase chain reaction (PCR) to generate individual fingerprints. Several conventional DNA fingerprinting probes were used to prime the PCR, yielding distinctive, hypervariable multifragment profiles for different strains of *Cryptococcus neoformans*. PCR fingerprinting with the oligonucleotide primers $(GTG)_5$, $(GACA)_4$, and the phage M13 core sequence (GAGGGTGGXGGXTCT), but not with $(CA)_8$ or $(CT)_8$, generated DNA polymorphisms with all 42 strains of *C. neoformans* investigated. PCR fingerprints produced by priming with $(GTG)_5$, $(GACA)_4$, or the M13 core sequence differentiated the two varieties of *C. neoformans*, *C. neoformans* var. *neoformans* (serotypes A and D) and *C. neoformans* var. *gattii* (serotypes B and C). Furthermore, strains of serotypes A, D, and B or C could be distinguished from each other by specific PCR fingerprint patterns. These primers, which also successfully amplified hypervariable DNA segments from other species, provide a convenient method of identification at the species or individual level. Amplification of polymorphic DNA patterns by PCR with these primers offers several advantages over classical DNA fingerprinting techniques, appears to be more reliable than other PCR-based methods for detecting polymorphic DNA, such as analysis of random-amplified polymorphic DNA, and should be applicable to many other organisms.

The identification of medically important fungi is based on morphological and physiological characteristics and is often difficult and time-consuming. Because the frequency of and mortality due to opportunistic mycoses are increasing among patients with AIDS, hematologic malignancies, and transplants, there is an urgent need for improved methods to identify fungal pathogens (15, 22). Novel molecular approaches for the genetic identification of fungal strains and species appear to offer advantages of simplicity, speed, and accuracy.

The basidiomycetous, encapsulated yeast *Cryptococcus neoformans* is among the most prevalent life-threatening mycotic agents. *C. neoformans* is found in the environment worldwide (15, 22), and inhalation of the yeast cells may lead to pneumonia or self-limited asymptomatic pulmonary infection. Although the overall incidence of cryptococcosis is relatively low, approximately 5 to 15% of patients with AIDS develop cryptococcal meningitis (4, 39). Two genetically distinct varieties of *C. neoformans* are recognized: *C. neoformans* var. *neoformans*, isolates of which have capsular polysaccharide serotypes that are designated A, D, or AD, and *C. neoformans* var. *gattii*, which is represented by strains of serotype B or C (2, 13, 14). Most cryptococcal infections are caused by strains of *C. neoformans* var. *neoformans* serotype A (15, 22), which are ubiquitous in soil and avian habitats. Strains of *C. neoformans* var. *gattii* are more common in tropical regions, such as southern California and western Australia, where they are found in association with eucalyptus trees (6, 7). Although globally most isolates are serotype A, strains of serotype A may vary in several pathobiological respects (3, 29), and the ability to identify individual strains would be advantageous.

Several studies have documented variation at the DNA level among strains of *C. neoformans*. Genetic differences were demonstrated by analysis of restriction fragment length polymorphisms in mitochondrial DNA (32). Chromosomal length polymorphisms were revealed by pulsed-field gel electrophoresis (26, 28). Analysis of restriction fragment length polymorphisms obtained by treating polymerase chain reaction (PCR)-amplified segments of the rRNA gene locus with restriction endonucleases distinguished several species of *Cryptococcus* but revealed few differences among strains of *C. neoformans* (23, 35). More recently, hybridization probes based on repetitive DNA sequences from *C. neoformans* were shown to distinguish strains of *C. neoformans* from other yeasts, such as *Candida albicans*, as well as to discriminate among strains of *C. neoformans* (27, 30, 33). Since these techniques can be laborious and time-consuming, they are not readily adaptable for routine diagnostic or epidemiological purposes.

Two methods have evinced considerable potential for the genetic identification of individual strains: DNA fingerprinting and random amplification of polymorphic DNA (RAPD). In 1985, Jeffreys et al. described the technique of DNA fingerprinting based on the detection of hypervariable repetitive DNA sequences by using core sequences from human repetitive DNA (12). In conventional DNA fingerprinting, Southern blots of genomic DNA are probed with various oligonucleotides to detect minisatellite or microsatellite DNA. This technique has since been used to identify individual genetic variability among closely related humans and

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TABLE 1. Strains of *C. neoformans* and related yeast species used in this study[a]

Species, variety, and strain (ATCC no.)[b]	Source	Serotype[c]	Original source or reference(s)
C. neoformans var. *neoformans*			
101	DUMC, J. R. Perfect	A	35
C3D	DUMC, J. R. Perfect	A	Clinical isolate; 35
H99	DUMC, J. R. Perfect	A	Spontaneous mutant of C3D
3501 (34873)	DUMC/ATCC, J. R. Perfect	D	From K. J. Kwon-Chung; 35
3502 (34874)	DUMC/ATCC, J. R. Perfect	D	From K. J. Kwon-Chung; 35
6 (62066)	T. G. Mitchell	A	Clinical isolate; 21
15 (62067)	T. G. Mitchell	A	Clinical isolate; 21
98 (62068)	T. G. Mitchell	A	From a cow; 21
110 (62069)	T. G. Mitchell	A	Clinical isolate; 21
145 (62070)	T. G. Mitchell	A	Clinical isolate from spinal fluid; 21
127.92	W. Schell		Environmental isolate from Duke campus
132.92	W. Schell		Environmental isolate from Duke campus
1988	DUMC, T. G. Mitchell		Clinical isolate from non-AIDS patient; 21
1218	DUMC, T. G. Mitchell		Clinical isolate from a non-AIDS patient; 21
1458	DUMC, T. G. Mitchell		Clinical isolate from a non-AIDS patient; 21
1.91	DUMC, T. G. Mitchell		Clinical isolate from a non-AIDS patient
1508	DUMC, T. G. Mitchell		Clinical isolate from an AIDS patient; 21
1948	DUMC, T. G. Mitchell		Clinical isolate from an AIDS patient; 21
1188	DUMC, T. G. Mitchell		Clinical isolate from an AIDS patient; 21
1018	DUMC, T. G. Mitchell		Clinical isolate from an AIDS patient
1958	DUMC, T. G. Mitchell		Clinical isolate from an AIDS patient
D321	DUMC, W. Schell		Clinical isolate; 35
n10	DUMC, J. R. Perfect		Clinical isolate from an AIDS patient; 35
n11	DUMC, J. R. Perfect		Clinical isolate from an AIDS patient; 35
n12	DUMC, J. R. Perfect		Clinical isolate from an AIDS patient; 35
n31	DUMC, J. R. Perfect		Clinical isolate from an AIDS patient; 35
n16	DUMC, J. R. Perfect		Busse-Bueske strain; 35
n18	DUMC, J. R. Perfect		Clinical isolate; 35
n25	DUMC, J. R. Perfect		Clinical isolate; 35
n27	DUMC, J. R. Perfect		Clinical isolate; 35
C. neoformans var. *gattii*			
n32	DUMC, J. R. Perfect	B	35
n33	DUMC, J. R. Perfect	C	35
n35	DUMC, J. R. Perfect	B or C	
371	UCLA, D. Howard	B	Clinical isolate from spinal fluid
373	UCLA, D. Howard	B	Clinical isolate
381	UCLA, D. Howard	B	From C. W. Emmons, NIH
385	UCLA, D. Howard	B	From J. E. Bennett, NIH
396	UCLA, D. Howard	B	Clinical isolate
380	UCLA, D. Howard	C	From E. E. Evans
381	UCLA, D. Howard	C	From C. W. Emmons, NIH
384	UCLA, D. Howard	C	Clinical isolate
385	UCLA, D. Howard	C	From J. E. Bennett, NIH
C. neoformans (variety unknown) 602	DUMC, T. G. Mitchell	None (no capsule)	From T. Kozel; 33, 35
C. albidus var. *albidus* (10666)	ATCC	None	Type culture; 35
C. albidus var. *diffluens* (12307)	ATCC	None	Type culture; 35
C. laurentii (18803)	ATCC	None	Type culture; 35
R. rubra (66034)	ATCC	None	

[a] Abbreviations: ATCC, American Type Culture Collection (Rockville, Md.); DUMC, Duke University Medical Center (Durham, N.C.); NIH, National Institutes of Health (Bethesda, Md.); UCLA, University of California at Los Angeles.

[b] The varietal status of each strain of *C. neoformans* was confirmed by growth on canavanine-glycine-bromthymol blue agar (16).

[c] Although strains of *C. neoformans* var. *neoformans* may be serotype A, D, or AD, on the basis of their clinical source and PCR fingerprinting, the untyped isolates are most probably serotype A. Strains of *C. neoformans* var. *gattii* are serotype B or C. Serotypes are indicated only for strains that were actually serotyped at the National Institutes of Health.

other animals and among plants and fungi (1, 8, 9, 11, 18–20, 25, 34). Useful probes for DNA fingerprinting include a number of cloned human repetitive DNA sequences (12), the core sequence of phage M13 (34), which detects minisatellite DNA, and synthetic oligonucleotides that detect microsatellite DNA (8). (Minisatellite DNA consists of sequences of repeated motifs of ca. 15 to 30 bp arranged in tandem at various loci [9], and microsatellite DNA is made up of motifs

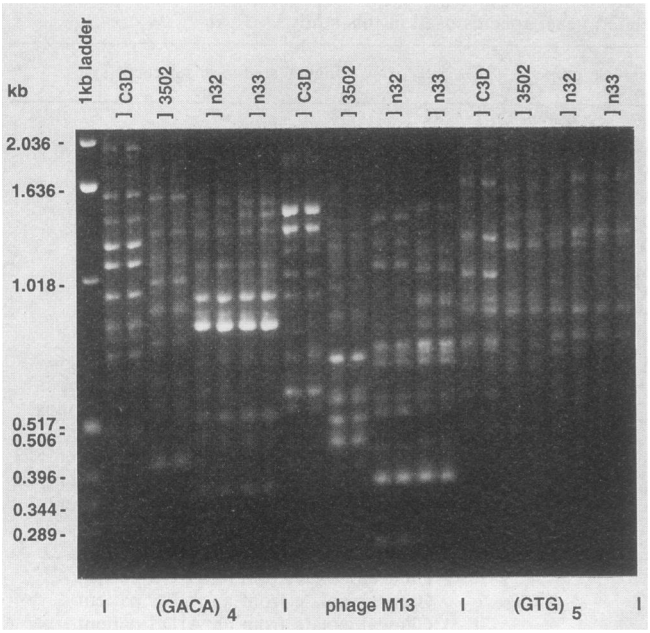

FIG. 1. Electrophoretic separation of PCR fingerprints obtained after amplification of genomic DNA of strains of *C. neoformans* with one minisatellite and two microsatellite probes as single primers: $(GACA)_4$, phage M13 core sequence (GAGGGTGGXGGXTCT), and $(GTG)_5$. In this comparison, each primer amplified duplicate preparations of the same four strains (indicated at the top): *C. neoformans* var. *neoformans* serotypes A (strain C3D) and D (strain 3502) and *C. neoformans* var. *gattii* serotypes B (strain n32) and C (strain n33). (These strains are listed in Table 1.)

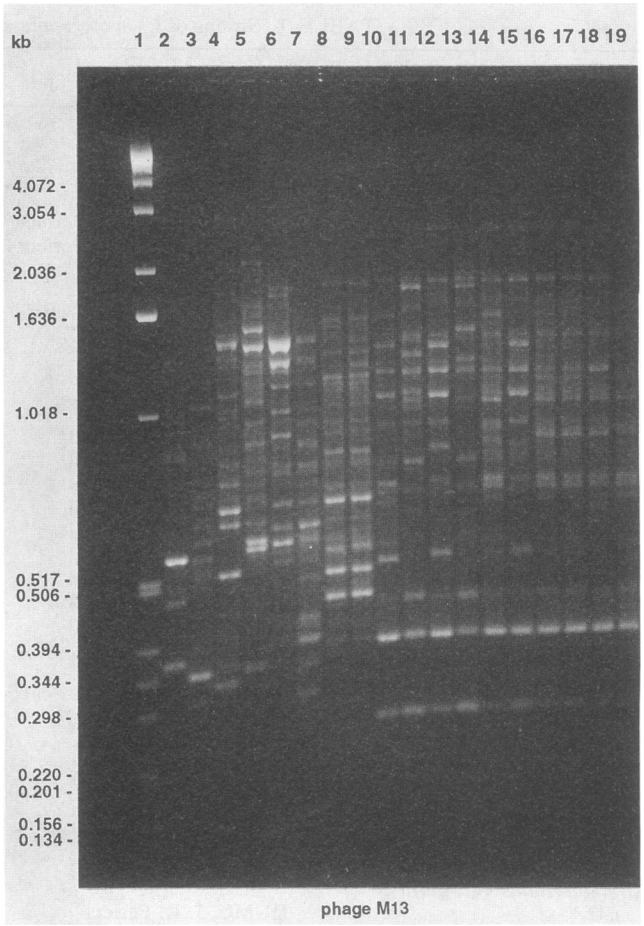

FIG. 2. Electrophoretic separation of PCR fingerprints obtained by amplifying genomic DNA from strains of *C. neoformans* and related species with the phage M13 core sequence (GAGGGTG GXGGXTCT) as the single primer. The template DNA contents of each lane are as follows, with the strain number in parentheses: lane 1, 1-kb ladder (GIBCO-Bio-Rad Laboratories); lane 2, *R. rubra* (ATCC 66034); lane 3, *C. laurentii* (ATCC 18803); lane 4, *C. albidus* var. *diffluens* (ATCC 12307); lane 5, *C. albidus* var. *albidus* (ATCC 10666); lanes 6 through 9, *C. neoformans* var. *neoformans* serotype A (strains C3D and 101) and serotype D (strains 3501 and 3502, respectively; lanes 10 through 15, *C. neoformans* var. *gattii* serotype B (strains n32, 371, 373, 381, 385, and 396, respectively); lanes 16 through 19, *C. neoformans* var. *gattii* serotype C (strains 380, 381, 384, and n33, respectively). (These strains are listed in Table 1.)

of only 2 to 10 bp arranged in tandem [31].) More recently, the technique of RAPD analysis for genetic characterization of organisms was described. In RAPD analyses, single primers of arbitrary nucleotide sequences of ca. 10 bp are used to randomly amplify polymorphic DNA fragments from different individuals (36, 38). An appropriate primer may yield distinctive patterns of DNA fragments with species or strain specificity. Because both DNA fingerprinting and RAPD analyses are capable of detecting variation among strains, we reasoned that a combination of the two techniques might yield a rapid, sensitive, and more reliable method, which would be applicable to large-scale experiments.

Oligonucleotides originally designed as hybridization probes for classical DNA fingerprinting experiments to detect minisatellite and microsatellite DNA were used as single PCR primers to amplify hypervariable DNA fragments in the genome of *C. neoformans* and closely related species. Although the PCR-amplified, hypervariable bands may not correspond to the satellite DNA detected with conventional DNA fingerprinting, the primers used were one that detects minisatellite DNA sequences, an oligonucleotide (GAGGGT GGXGGXTCT) of the core sequence from the phage M13 (34), and primers that detect microsatellite DNA sequences, namely, $(CA)_8$, $(CT)_8$, $(GTG)_5$, and $(GACA)_4$ (1, 8, 24). The electrophoretic profiles that resulted from amplification with three of these primers, $(GTG)_5$, $(GACA)_4$, and the M13 core sequence, were highly reproducible and exhibited variation at the species, subspecies (variety), and individual strain levels. The other two primers, $(CA)_8$ and $(CT)_8$, did not amplify DNA from any of the strains under the same conditions.

MATERIALS AND METHODS

Yeast strains. A total of 42 clinical and environmental isolates of *C. neoformans*, including several from patients with AIDS, and three related yeast species, *Cryptococcus albidus*, *Cryptococcus laurentii*, and *Rhodotorula rubra*, were studied (Table 1). Cultures of these yeasts were grown in yeast nitrogen base medium (Difco, Detroit, Mich.), supplemented with 1% (vol/vol) glucose and 1.5 g of asparagine per liter, at 30 or 37°C until they reached the stationary phase, when the DNA was isolated.

DNA isolation. DNA was similarly isolated from small-scale (1-ml) and larger-scale (200-ml) cultures, as follows. Stationary-phase yeast cells were pelleted by centrifugation and washed three times in cold buffer (20 mM sodium citrate [pH 5.8] in 1 M sorbitol), ground in liquid nitrogen (in a biosafety cabinet), and resuspended in lysis buffer (50 mM

Tris-HCl [pH 7.2], 50 mM EDTA, 3% sodium dodecyl sulfate, and 1% β-mercaptoethanol). The suspension was then incubated at 65°C for 1 h. After the cell debris had been pelleted, the lysate was extracted once with phenol-chloroform (1:1) and once with a chloroform-isoamyl alcohol (24:1). The DNA was precipitated by the addition of 0.2 volume of 3 M sodium acetate and 0.5 volume of isopropanol; after centrifugation, the DNA pellets were washed with 70% ethanol and resuspended in buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). Samples of DNA from the larger culture volumes were further purified by standard CsCl gradient centrifugation (17).

Primers. The following primers were used: the microsatellite DNA sequences $(CA)_8$, $(CT)_8$, $(GTG)_5$, and $(GACA)_4$ (1, 8, 24) and the phage M13 core sequence, GAGGGTG GXGGXTCT (34).

PCR. Amplification reactions were performed with volumes of 50 μl containing 10 to 25 ng of genomic DNA, 3 mM magnesium acetate, 0.2 mM each deoxynucleoside triphosphate (dNTP) (U.S. Biochemicals, Cleveland, Ohio), 20 to 30 ng of primer, and 2.5 U of Amplitaq DNA polymerase (Perkin-Elmer Cetus). Under the recommended buffer conditions, the PCR was performed for 40 cycles in a Perkin-Elmer Cetus DNA thermal cycler (model 480) as follows: 20 s of denaturation at 93°C, 60 s of annealing at 50°C, and 20 s of primer extension at 72°C, followed by a final extension cycle for 6 min at 72°C.

Analysis of PCR fingerprints. Amplification products were analyzed by electrophoresis in 1.4% agarose gels run in Tris-borate-EDTA buffer (17) for ca. 7 h and detected by staining with ethidium bromide under UV light. Electrophoretic bands were sized and compared with a SparcStation scanner and commercial software (The Discovery Series; PDI, Huntington Station, N.Y.).

RESULTS

The primers $(GTG)_5$ and $(GACA)_4$ and the M13 core sequence primer each successfully amplified variable DNA fragments from all the strains of *C. neoformans*, *C. albidus*, *C. laurentii*, and *R. rubra* (Table 1). The patterns generated with each of these primers were distinctive and highly reproducible (Fig. 1 and 2). The primers $(CA)_8$ and $(CT)_8$ did not amplify DNA from any of the strains tested. Because minor impurities might affect amplification, PCR fingerprints were generated from both crude and CsCl-purified preparations of genomic DNA from the same yeast strains, and the resulting fingerprint patterns were comparable. Since the two DNA preparations gave essentially the same PCR fingerprint pattern, crude DNA minipreps appear to be adequate for routine PCR fingerprinting. Although the intensities of individual bands sometimes varied among replicate PCR fingerprints of a given strain, the position of each band was always the same (data not shown). Others have also observed occasional variation in the intensity of bands in duplicate PCR experiments (5, 10).

The sizes of the amplification products ranged from 0.2 to 2 kb. The number of bands obtained in the PCR fingerprint pattern depended on the primer that was used. With DNA from these yeasts, primer $(GTG)_5$ usually produced the fewest bands (6 to 17), $(GACA)_4$ yielded 10 to 20 bands, and the M13 core sequence primer generated 14 to 38 bands.

The PCR fingerprints produced by these three primers (Fig. 1 and 2) clearly distinguished variation among strains of *C. neoformans* at three levels: species, variety, and individual. At the species level, the PCR fingerprint patterns of the strains of *C. albidus*, *C. laurentii*, and *R. rubra* clearly differed from each other and from those of all of the *C. neoformans* isolates (Fig. 2). Characteristic genetic variation was also observed among varieties and serotypes of *C. neoformans*. Figure 1 depicts the PCR fingerprints of strains that represent both varieties of *C. neoformans* and each of the four serotypes. Serotypes A and D of *C. neoformans* var. *neoformans* revealed two quite different fingerprint patterns. In contrast, the patterns produced by serotype B and C strains of *C. neoformans* var. *gattii* were indistinguishable. Indeed, each primer yielded three general patterns of PCR fingerprints (Fig. 1); one pattern corresponded to serotype A (strain C3D), one corresponded to serotype D (strain 3502), and the third corresponded to serotypes B and C (strains n32 and n33). Each of these general patterns was characterized according to the major bands (i.e., the intense bands com-

TABLE 2. Molecular sizes of major identifying or diagnostic DNA bands of each serotype of *C. neoformans*, obtained with various primers

Primer	*C. neoformans* serotype	No. of major diagnostic bands	Molecular sizes of major bands (kb)[a]
$(GACA)_4$	A	10	1.998, 1.570, 1.383, 1.298, 1.212, 1.098, 0.946, 0.821, 0.783, 0.495
	D	9	1.559, 1.302, 1.123, 1.013, 0.884, 0.763, 0.633, 0.565, 0.425
	B or C	8	1.523, 1.430, 1.302, 1.101, 0.940, 0.826, 0.541, 0.371
M13	A	10	1.448, 1.332, 1.191, 1.054, 0.951, 0.826, 0.811, 0.712, 0.606, 0.563
	D	13	1.619, 1.369, 1.282, 1.209, 0.987, 0.866, 0.707, 0.601, 0.585, 0.529, 0.470, 0.415, 0.400
	B or C	10	1.974, 1.651, 1.408, 1.231, 1.104, 0.732, 0.550, 0.457, 0.392, 0.275
$(GTG)_5$	A	11	1.897, 1.682, 1.275, 1.209, 1.095, 1.057, 0.895, 0.763, 0.703, 0.601, 0.492
	D	7	1.631, 1.405, 1.212, 0.946, 0.884, 0.754, 0.689
	B or C	8	1.706, 1.288, 1.228, 0.886, 0.785, 0.732, 0.589, 0.574

[a] Molecular sizes of the DNA bands were determined automatically from computer-scanned photographic negatives of agarose gels by comparison with molecular size standards (The Discovery Series; PDI). Analysis involved 26 strains of serotype A, 2 of serotype D, and 12 of serotype B or C.

TABLE 3. Degree of band sharing between pairs of strains of *C. neoformans* of each serotype

Primer	*C. neoformans* serotypes compared	No. of strains compared	Average no. of bands	Average no. of common bands	*S* value[a]
$(GACA)_4$					
	A/A	26/26	12.2	9	0.74
	B/B	6/6	15	13	0.86
	C/C	6/6	15	13	0.86
	D/D	2/2	16.5	15	0.90
	A/B	26/6	13.6	0	0
	A/C	26/6	13.6	0	0
	A/D	26/2	14.3	1	0.06
	B/C	6/6	15	13	0.86
	B/D	6/2	15.7	1	0.06
	C/D	6/2	15.7	1	0.06
M13 (GAGGGTGGXGGXTCT)					
	A/A	26/26	23.4	18	0.76
	B/B	6/6	25.8	17	0.68
	C/C	6/6	28.4	21	0.74
	D/D	2/2	28.5	24	0.84
	A/B	26/6	24.6	1	0.04
	A/C	26/6	25.9	1	0.04
	A/D	26/2	25.9	2	0.08
	B/C	6/6	25.7	18.6	0.72
	B/D	6/2	25.6	3	0.12
	C/D	6/2	28.4	3	0.10
$(GTG)_5$					
	A/A	26/26	11.2	9	0.80
	B/B	6/6	13	10	0.77
	C/C	6/6	10	9	0.90
	D/D	2/2	11	9	0.81
	A/B	26/6	12.1	6	0.49
	A/C	26/6	10.6	6	0.56
	A/D	26/2	11.1	2	0.18
	B/C	6/6	11.5	9	0.78
	B/D	6/2	12	3	0.25
	C/D	6/2	10.5	3	0.28

[a] Band-sharing coefficients were calculated as $S = 2N_{AB}/(N_A + N_B)$, where N_A and N_B are the total number of bands for the strains to be compared and N_{AB} represents the number of common bands (37).

mon to each strain) that were typical of the corresponding serotype(s) (Table 2). These results suggest that the two serotypes of *C. neoformans* var. *neoformans* are genetically distinct. Other investigators have also noted genetic differences among serotypes (26, 28, 30, 33). Strains of *C. neoformans* var. *gattii* (serotypes B and C) were more genetically homogeneous than *C. neoformans* var. *neoformans* strains (serotypes A and D) (Fig. 2 and Table 3; see below). We also observed smaller bands that varied among individual strains and were strain specific. Indeed, the profiles of no two (or more) strains were completely identical.

The degree of relatedness among strains can be calculated in terms of band sharing or similarity coefficients (*S* values), as proposed by Wetton et al. (37). Strains of a given serotype had coefficients between 0.7 and 0.9, indicating their close similarity (Table 3). However, pairwise comparisons of strains of different serotypes yielded *S* values that were much lower, except in comparisons of serotypes B and C. The greatest distinction among serotypes was achieved with the PCR fingerprint patterns generated by the $(GACA)_4$ and M13 sequence primers. With both of these primers, calculations of the *S* values indicated a very low degree of similarity among the different serotypes ($S \leq 0.12$). With the oligonucleotide $(GTG)_5$, the *S* values showed a higher degree of similarity among strains of different serotypes (*S* values between 0.18 and 0.56).

DISCUSSION

The application of conventional DNA fingerprinting hybridization probes as single primers in the PCR combines the advantages of DNA fingerprinting with those of the PCR. The high degree of DNA polymorphism detected by conventional multilocus probes in DNA fingerprinting experiments is combined with the technical simplicity and speed of the PCR method, facilitating large-scale experiments. The conventional fingerprint procedure entails extraction of genomic DNA, usually by the CsCl method, digestion with restriction enzyme(s), separation of the DNA fragments by agarose gel electrophoresis, denaturation, blotting of single-stranded DNA to a membrane, hybridization of the membrane-bound DNA with a labeled oligonucleotide probe, washing, and detection of bands by autoradiography or a nonradioactive label. In contrast, the PCR procedure involves only extraction of genomic DNA (by CsCl or rapid minipreparation), amplification with the oligonucleotide, separation of the PCR products by agarose gel electrophoresis, and analysis of photographed bands.

PCR fingerprinting is demonstrated here by comparing strains of *C. neoformans* representing both varieties and the four serotypes. The procedure is highly reproducible, and as observed with *C. neoformans* (Fig. 2) and *Candida* species (unpublished data), strains of a species can be readily

distinguished by species- and strain-specific bands. PCR fingerprinting has a greater discriminatory power than electrophoretic karyotyping. Recent data showed that several strains of *C. neoformans* from different geographic locations possessed the same karyotype (25a) but were distinguishable by PCR fingerprinting (unpublished data). We are currently determining the sequences of several dominant PCR fingerprint fragments (Table 2).

Reproducible fingerprint patterns require standardized conditions, such as the same concentrations of reagents (e.g., buffer, dNTPs, and magnesium acetate) and the same thermal cycler and cycling conditions. Slight variations in conditions may explain the occasional variations in band intensities. We have observed some interlaboratory variability (unpublished observation), but when the same cycler, reagents, and electrophoretic and other conditions are repetitively tested within a laboratory, multiple tests of an individual strain generate identical PCR fingerprints. We are also investigating the stability of PCR fingerprints after storage, exposure to antifungal drugs, or passage through animals.

Strains of serotypes B and C were nearly indistinguishable, suggesting less genetic variability than among strains of serotypes A and D. Depending on the primer, the *S* values for serotypes B and C of strains of *C. neoformans* var. *gattii* varied from 0.72 to 0.86. However, using classical DNA fingerprinting and a different probe, as well as a larger number of isolates of serotypes B and C, Varma and Kwon-Chung observed greater variation among serotypes B and C than among serotypes A and D (33). We intend to collect and examine many more strains of each serotype from a variety of sources. With all three primers used here, DNA from the nonencapsulated strain 602 generated a PCR fingerprint pattern similar to that of strains of serotype A (data not shown), which was also reported by Varma and Kwon-Chung (33).

The method described here also provides additional information for improving the established systems of classification of medically important fungi. For example, it is now convenient to obtain DNA data about the serotype of any *C. neoformans* isolate along with a strain-specific PCR fingerprint band pattern. This technique should be able to be used to answer many important epidemiological questions concerning the distribution and dispersal of genetically distinct isolates.

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